dinium isothiocyanate phenol/chloroform (AGPC) method[2]. First-strand cDNA synthesis was performed by use of murine leukemia virus reverse transcriptase (MLV RT) or RNaseH-reverse transcriptase and either random hexamers or an 12 mer oligonucleotide primer (5′-TAGTAGTAGACT-3′) complementary to 3′-ends of all three hantaviral RNA segments[6]. The optimal reaction conditions, annealing temperature and thermocycle profile were determined in sequential experiments using cDNA synthesized from RNA of Hantaan-infected Vero E6 cells. Of numerous S and M segment primers tested one genus-reactive S segment primer pair consistently gave the best results with different hantaviruses. This primer pair was designed to match conserved regions of all hantaviral serotypes, degenerated in 3′ positions to fit to all known hanta sequences (sense primer S1: 5′-GG(AC)CAGACAGCAGA(CT)TGG-3′ antisense primer S2: 5′-AGCTCAGGATCCAT(AG)-TCATC-3′. Two internal primer pairs specific for HTN/SEO and PUU were used for second-round (nested) PCR, resulting in a 376 bp or 304 pb amplification product, respectively. The identity of the products was verified by hybridization, RFLP or sequencing after cloning in appropriate vectors.

Although several patients with suspected HFRS infections were analyzed using this RT-PCR method, only in one case of a patient with acute multi-organ failure of unknown aetiology could a positive PCR reaction in peripheral blood leukocytes (PBL) be determined. This is in general agreement with results of other workers, indicating that hanta RNA in PBL could only be detected early in the course of disease[1].

Recently we were able to amplify hantavirus sequences from a lung tissue specimen of a vole (*Microtus arvalis*) that was also characterized by a positive reaction in hantavirus enzyme immunoassay. The vole was trapped in early 1994 near Malacky, Slovakia, a geographical area where HFRS is endemic. Cloning and subsequent sequence analysis of a 840 bp region in the S segment revealed a novel hantavirus sequence, which led us to conclude that we had found a new hantavirus type.[5]

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Amplification and detection of enterovirus RNA and herpes virus DNA in CSF samples by multiplex polymerase chain reaction

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Herpes simplex virus (HSV) is a common cause of sporadic, acute focal encephalitis. Without early institution of antiviral therapy, death or severe neurologic impairment results in most cases. Enteroviruses are the most commonly identified causes of aseptic meningitis but have also been found in cases of acute encephalitis. Enteroviral CNS infections usually resolve without sequelae. A specific treatment is not yet available. However, herpes simplex virus encephalitis (HSVE) and enteroviral encephalitis are clinically indistinguishable. For a rapid diagnosis of HSVE and of enteroviral CNS infection, the polymerase chain reaction has been employed to detect HSV DNA and enteroviral RNS in CSF samples[1,2,4]. Recently, the simultaneous amplification of HBV and HCV genomic sequences in human serum samples has been described[3]. HCV RNA and HBV DNA were prepared from the samples by two different methods. We tested a simple, commercially available extraction method for the simultaneous isolation of DNA and RNA in one fraction.

Nucleic acids were extracted from 130 µl of human CSF samples and from control samples by the QIAamp HCV Kit (Qiagen) according to the instructions of the manufacturer. Nucleic acids were eluted from the QIAamp column in 50 µl H_2O. Ten microlitres of the nucleic acid eluate were reverse transcribed to cDNA. Enteroviral cDNA and HSV DNA were co-amplified using primer pairs from the 5′-UTR of the enteroviral genome[2,5] and of the HSV glycoprotein B region[1,4]. PCR products were detected by agarose gel electrophoresis and ethidium bromide staining. The specificity of the products was confirmed by a second round of amplification using nested primer pairs.

Positive control samples were spiked with echovirus 6 and with a plasmid containing the HSV glycoprotein B sequence. Nucleic acid extraction and co-amplification yielded two products of the expected sizes. Sensitivity was <100 copies for the HSV plasmid and <0.1 pfu for echovirus 6.

Several clinical samples of patients known to have HSVE or enteroviral CNS infections were tested by the

multiplex PCR protocol and found to be positive for HSV DNA or enterovirus RNA, respectively. Control samples of patients with other CNS infections were found to be negative.

In conclusion, we have described a sensitive and specific procedure to amplify simultaneously enteroviral and HSV genomic sequences in human CSF samples. The multiplex PCR for HSV DNA and enteroviral RNA provides a means to detect HSV or enteroviral genomic sequences without the need for separate extraction or amplification procedures. The rapid differential diagnosis between HSV and enteroviral CNS infection should be of value for therapeutic decisions and prognostic evaluation.

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Evaluation of two RT/PCR assays for HCV by testing blood of intravenous drug abusers

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A highly sensitive and specific RT/double PCR assay with heminested primers was developed to detect hepatitis C virus (HCV) RNA in blood specimens. The performance of this tailored assay and of a commercially available RT-PCR assay (HCM-Amplicor™) were compared by analysis of a coded panel (EUROHEP II) of samples. Both assays proved to be highly reliable with respect to specificity. Although the tailored assay was 1.6 times more sensitive, overall performance of both tests appeared to be similar. To assess the diagnostic utility of the assays, the prevalence of HCV in a population of intravenous drug users (IVDU) was investigated. Both assays appeared to be suitable for testing and showed that IVDU are a group with high prevalence of circulating HCV.

Methods and results. To assure highest technical standards of PCR and to avoid carry-over and cross-contamination the following measures were taken: i) one way flow of technicians and material through four separate laboratory rooms, ii) double testing, iii) random processing of samples, iv) interspersed negative controls for every two clinical samples. Samples with discordant results in one or among the two HCV PCR assays were retested.

Both HCV PCR assays employed oligonucleotide primers directed to similar regions of the highly conserved 5′-non translated region (5′-NTR) of the HCV genome[1]. Oligonucleotides for the tailored assay were the A-oligonucleotides of Bukh et al.[1,2]. Pre-PCR sample extraction for the Amplicor™ was according to the manufacturer. In contrast, plasma was merely denatured by heat in the presence of 0.4% Nonidet-P 40 before being subjected to the tailored PCR. Specificity for HCV of this latter assay had been documented by inclusion of known positive and negative controls and by characterization of amplicons I and II by size, restriction fragment analysis and by amplification with heminested primers. The assay sensitivities of both PCRs were determined by use of a human serum, the infectious titre of which had been measured by inoculation of chimpanzees (H-strain of HCV, kindly donated by Dr. R. H. Purcell, NIH, Bethesda, USA). The direct sensitivity of both assays was in the range of 10 to 100 CID_{50} (50% chimp infectious doses). However, because the tailored assay utilized only 3 µl of plasma instead of a 5 µl equivalent of a 100 µl specimen for the commercial assay, it was 1.6 times more sensitive.

The diagnostic sensitivities of both tests were evaluated on 26 coded samples provided by the EUROHEP study group. Neither test resulted in false positives or false negatives. On one serially diluted sample the tailored PCR was four times more sensitive than the Amplicor™ and than one of two reference assays. However, with a different sample the commercial PCR proved four times more sensitive than the tailored assay. Hence, both tests appeared to be similarly suitable for general testing.

The clinical/diagnostic performances of the two HCV PCRs were assessed by investigation of the virus' prevalence among 114 IVDU who had enrolled in a methadone programme. In addition to prevalence of HCV, the seroprevalences of hepatitis B and C virus were determined (IMX-HBV, HCV-Matrix, Abbott). Among the 114 samples tested, results of only 4 (3.5%) were discordant between the two HCV PCRs and remained so after repeat testing.

Infections by HCV and HBV had been experienced by 72 and 65% of patients, respectively. In 60% of anti-HCV positive cases, circulating virus was detected. This high prevalence of HCV is well in accordance with the high probability (60 to 80%) that infections will progress to chronicity. Furthermore, the investigation showed that IVDU patients present as a high